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Clinical and Molecular Virological Differences Between Fulminant Hepatic Failures Following Acute and Chronic Infection With Hepatitis B Virus

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Clinical and molecular biological characteristics were compared between patients who presented with fulminant hepatic failure following acute infection with hepatitis B virus (HBV) and those who developed hepatic failure during they carried HBV. The 11 patients with acute HBV infection had higher levels of alanine aminotransferase (mean ± SD: 4943 ± 2867 vs. 1157 ± 678 IU/L, $P < 0.01$), more often with a single peak (91% vs. 0%, $P < 0.001$), and lower total bilirubin levels (15.3 ± 4.4 vs. 28.1 ± 14.3 mg/100 ml, $P < 0.01$) than the 13 patients with chronic HBV infection. Hepatitis B surface antigen was detected less often (55% vs. 100%, $P < 0.05$) and viral DNA polymerase less frequently (0% vs. 46%, $P < 0.05$) in the patients with acute than chronic HBV infection. Hepatitis B e antigen was detected in one (9%) patient with acute infection, less frequently than in six (46%) patients with chronic infection ($P < 0.05$). Mutations in the precore region was detected in HBV DNA clones from ten (91%) patients with acute infection and only in those from eight (62%) patients with chronic infection. All HBV DNA clones from the five (38%) patients with chronic infection that did not have precore mutations, however, possessed mutations in the core promoter. These results indicate that HBV mutants incapable of translating hepatitis B e antigen would play a major role in fulminant hepatic failure occurring after acute HBV infection. In contrast, HBV variants with core promoter mutations for reducing the transcription of hepatitis B e antigen would play an additional role in fulminant hepatic failure developing during chronic infection. ***J. Med. Virol. 55: 35–41, 1998.***

KEY WORDS: fulminant hepatic failure; hepatic encephalopathy; hepatitis B virus; hepatitis B e antigen; DNA mutational analysis; promoter regions

INTRODUCTION

Hepatitis B virus (HBV) can cause fulminant hepatic failure (FHF). In Western countries where HBV is not endemic, FHF develops mostly after acute infection with HBV [Saracco et al., 1988], although severe exacerbation is reported in some patients with chronic hepatitis B [Davis and Hoofnagle, 1985]. In areas where HBV is endemic such as Asia, however, FHF develops more frequently in the individuals who have carried HBV for a long time without symptoms of hepatitis [Chu and Liaw, 1990]. Superinfection of HBV carriers with the other hepatitis viruses, such as hepatitis delta virus and hepatitis C virus (HCV), is implicated in FHF occurring in some HBV carriers [Saracco et al., 1988; Wu et al., 1994].

It is not clear if there are any clinical and virological differences between FHF occurring after acute HBV infection and that developing during chronic HBV infection. Should there be any differences between FHF occurring after acute HBV infection and that developing during chronic HBV infection, they would be useful for accurate diagnosis and effective treatment of this most severe form of viral hepatitis.

Clinical, serological and molecular virological characters of FHF were compared between the 11 patients who presented with fulminant hepatic failure after acute infection and the 13 patients who developed FHF while they carried HBV, none of whom had evidence of other hepatitis virus infections.

MATERIALS AND METHODS

Patients

During a period from 1986 to 1994, 24 patients were admitted to Showa University Fujigaoka Hospital for the treatment of FHF due to HBV infection. Eleven were considered to have acute HBV infection based on high titres of antibody to hepatitis B core antigen (anti-

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HBc) of IgM class and no previous history of hepatitis. The remaining 13 patients had been persistently positive for hepatitis B surface antigen (HBsAg) in serum and followed-up as outpatients for 3–5 years until they developed FHF; they all had high IgG but low IgM anti-HBc titres. They were symptom-free with occasional moderate elevation in ALT until the development of FHF; liver biopsy was not undertaken due to the benign nature of the carrier state. Five patients with chronic HBV infection had received intensive chemotherapy for the treatment of non-Hodgkin lymphoma and another had received steroid therapy; FHF developed after the withdrawal of therapy in these patients. Co-infection with hepatitis A virus, HCV or hepatitis delta virus was excluded from all of the patients studied.

The diagnosis of FHF was contingent on: (a) the development of stage II–IV hepatic encephalopathy within 8 weeks of the onset of illness; (b) a prothrombin <40%; and (c) no known history of previous liver diseases to exclude chronic liver failure. They were all treated with the artificial liver support system that has been developed in Showa University Fujigaoka Hospital; consisting of plasma exchange and haemodiafiltration with high performance membranes in a bicarbonate buffer [Yoshiba et al., 1993]. Markers of HBV infection were sought in sera from patients. HBV DNA clones were propagated from sera and sequenced within the precore region as well as the core promoter [Yuh et al., 1992]. The study was approved by the Ethics Committee of the hospital, and the informed consent was obtained from patients or, when they were unable to make a decision, from their family members serving as legal guardians.

Markers of Hepatitis Virus Infection

HBsAg and the corresponding antibody (anti-HBs), hepatitis B e antigen (HBeAg) and antibody to it (anti-HBe), and IgM anti-HBc were determined by radioimmunoassay with commercial kits (Ausria, Ausab, HBeAg-RIA and CORAB-M: Abbott Laboratories, Illinois, USA). IgM anti-HBc was determined in sera diluted 1000-fold for detecting only high-titered antibodies. Antibody to hepatitis delta virus and IgM antibody to hepatitis A virus were determined using commercial assay kits (HAVAB-M · EIA and ANTI-DELTA · RIA: Abbott Laboratories). Antibody to HCV was determined by enzyme immunoassay of the second generation (EIA-II, Ortho Diagnostic Systems, Tokyo, Japan). The activity of HBsAg-associated DNA polymerase was determined by the method of Kaplan et al. [1973].

Determination of HBV DNA

Serum (0.1 ml) was treated with proteinase K and sodium dodecyl sulphate, and nucleic acids were extracted with phenol-chloroform by the method described previously [Okamoto et al., 1990]. HBV DNA was amplified on extracted nucleic acids by polymerase chain reaction (PCR) with nested primers deduced from the S gene [Iizuka et al., 1992].

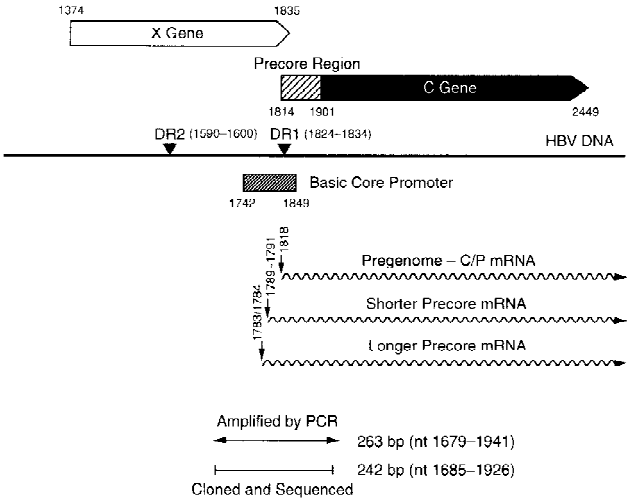

Fig. 1. A map of HBV DNA with the location of amplified and sequenced fragment. The X gene and the core gene with the precore region are indicated by boxes with arrow heads. The basic core promoter is indicated by a hatched box. Three genomic mRNAs of 3.5 kilobases are shown in the middle. They have different transcription start sites within the basic core promoter and share the 3′ end. Triangles indicate positions of direct repeats (DR1 and DR2). Nucleotide fragment amplified by PCR with nested primers is indicated below. A major part of it indicated by a line with vertical bars at both ends was cloned and sequenced.

Sequencing a Part of the X Gene and the Precore Region

This was undertaken by the method described elsewhere [Okamoto et al., 1994]. Briefly, HBV DNA was extracted from serum and a fragment of 263 base pairs (bp) corresponding to nt 1679–1941 was amplified, by PCR with nested primers, which included the core promoter spanning nucleotides (nt) 1742–1849 [Yuh et al., 1992] and the precore region (nt 1814–1900) as illustrated in Figure 1. HBV DNA amplified by PCR was digested with *Eco*RI and *Hin*cII (TaKaRa Biomedicals, Kyoto, Japan). The digest of 242 bp spanning nt 1685–1926 was ligated to the M13 phage vector that had been cleaved with *Eco*RI and *Hin*cII, and clones bearing HBV DNA sequences were propagated. HBV DNA sequences were then determined by the dideoxy-chain termination method with the Sequenase DNA sequencing kit (7-deaza-dGTP edition version 2.0, United States Biochemical Corp., Ohio, USA) or the AutoRead DNA sequencing kit (Pharmacia LKB Biotechnology, Uppsala, Sweden).

Statistical Analyses

Frequency between groups was compared using the χ^2 test and the Fisher's exact test, and group means were compared using the Welch's *t*-test.

RESULTS

Clinical and Biochemical Features of the Patients with FHF

Table I compares features of the 11 patients who presented with FHF after acute HBV infection and the

TABLE I. Clinical and Biochemical Findings in Patients Who Developed Fulminant Hepatic Failure after Acute or during Chronic HBV Infection

Features	HBV infection: Acute (n = 11)	HBV infection: Chronic (n = 13)	Differences
Age (years)	51.9 ± 16.6	46.6 ± 12.4	
Male, n (%)	8 (73 [44–92][e])	9 (69 [43–89])	
Days from jaundice to coma	2.8 ± 1.5	34.2 ± 46.4	$P < 0.05$
Survival, N (%)	8 (73 [44–92])	5 (39 [16–65])	
ALT[a] (IU/L)	4943 ± 2867	1157 ± 678	$P < 0.01$
AST[b] (IU/L)	6611 ± 3283	1295 ± 939	$P < 0.001$
Peaks of ALT			
Single n (%)	10 (91 [64–100])	0 (0 [0–21])	$P < 0.001$
Multiple n (%)	1 (9 [0–36])	13 (100 [79–100])	$P < 0.001$
Bilirubin			
Total (mg/100 ml)[c]	15.3 ± 4.4	28.1 ± 14.3	$P < 0.01$
Direct/total ratio[d]	0.48 ± 0.26	0.58 ± 0.13	
Prothrombin (%)	14.7 ± 11.5	20.8 ± 7.7	

[a]Alanine aminotransferase (normal values: <40 IU/L).
[b]Aspartate aminotransferase (normal values: <40 IU/L).
[c]Maximal values.
[d]Minimal values.
[e]A 95% confidence interval is shown in brackets.

13 patients who developed FHF while they carried HBV. The patients with acute infection, diagnosed by high-titered IgM anti-HBc in serum, ran a more drastic course. They developed coma earlier after the manifestation of jaundice and had higher maximal transaminase levels than the patients with chronic infection. However, the maximal level of total bilirubin was lower in the patients with acute than chronic HBV infection. Most patients with acute HBV infection experienced a single peak of elevated alanine aminotransferase (ALT), while multiple bouts of elevated ALT were the rule in those with chronic infection. Falling short of being significant, the survival rate was higher and prothrombin levels lower in the patients with acute than chronic HBV infection. Taken together, symptoms of FHF would be more severe but the chances for survival better with the artificial liver support system [Yoshiba et al., 1993] in the patients with acute than chronic HBV infection.

HBV Markers in Patients Who Developed FHF after Acute or during Chronic HBV Infection

Table II compares various HBV markers in the two groups of patients with FHF. IgM anti-HBc was detected in high titres in all the 11 patients with acute infection, but in only one of the 13 patients with chronic infection in a lower titre. HBsAg was detected in six of the 11 (55%) patients with acute infection at the onset of disease and became undetectable in five within 1 week after coma. The remaining patient (Case 10) had been on maintenance haemodialyisis and kept HBsAg for 20 days until death. By contrast, HBsAg was positive throughout the course in all the 13 patients with chronic infection. Thus, the persistence of HBsAg was more common in the patients with chronic than acute infection (100% vs. 9%, $P < 0.001$). HBeAg was detected in only one (9%) patient with acute infection, which was less frequent than the detection in six (46%) patients with chronic infection ($P < 0.05$).

HBsAg-associated DNA polymerase was not detected in any patient with acute infection, while it was positive in six (46%) patients with chronic infection at the onset of FHF. HBV DNA was detected by PCR in sera from all patients when they presented with FHF. Within 2 weeks after the onset, however, HBV DNA became undetectable in some; it was detected only in one (9%) patient with acute infection less frequently than in ten (77%) patients with chronic infection ($P < 0.001$).

Molecular Virological Analysis of HBV

From serum of each patient with FHF, 3–24 independent HBV DNA clones were propagated, and the core promoter and precore region were sequenced (Fig. 1). The various mutations observed are listed in Table III. Due to small numbers of clones tested in some patients, mutations in minor populations of HBV may have been missed.

The G-to-A point mutation at nt 1896 (A1896), converting codon 28 in the precore region for tryptophan to a stop codon, was detected in all 108 HBV DNA clones from ten (91%) patients with acute infection. The remaining one patient (Case 11) was positive for HBeAg and did not have A1896; a 7-bp insertion after nt 1825 that induced a frame shift was detected in one of the 8 clones from him. By contrast, A1896 was observed only in eight (62%) patients with chronic HBV infection. It was detected in all HBV DNA clones from six patients and in approximately half the clones from the remaining two patients (Cases 6 and 7).

Mutations in the core promoter were detected in all HBV DNA clones from 12 (92%) patients with chronic HBV infection and six (55%) patients with acute infection. Core promoter mutations were not accompanied

TABLE II. Viral Markers in Patients who Developed Fulminant Hepatic Failure after Acute or during Chronic HBV Infection

Patient number	Age and sex	Outcome	HBsAg	HBeAg or anti-HBe	IgM anti-HBc[a]	HBV DNA polymerase[b] (cpm/ml)
After Acute HBV Infection						
1	21F	Survived	–	Anti-HBe	+	–
2	38F	Survived	+	Anti-HBe	+	–
3	43M	Survived	+	Anti-HBe	+	–
4	45F	Survived	+	Anti-HBe	+	–
5	47M	Survived	–	Anti-HBe	+	–
6	51M	Survived	–	Anti-HBe	+	–
7	54M	Survived	–	Anti-HBe	+	–
8	53M	Died	–	Anti-HBe	+	–
9	67M	Died	+	Anti-HBe	+	–
10	82M	Died	+	Anti-HBe	+	–
11	70M	Survived	+	HBeAg	+	–
During Chronic HBV Infection						
1	31M[c]	Died	+	HBeAg	–	272
2	35M	Survived	+	HBeAg	–	357
3	40M[d]	Survived	+	Anti-HBe	–	–
4	52F	Died	+	HBeAg	+	–
5	54F	Survived	+	HBeAg	–	1,564
6	39M	Died	+	Anti-HBe	–	–
7	31M	Survived	+	HBeAg	–	–
8	41M	Died	+	Anti-HBe	–	–
9	46M[d]	Died	+	Anti-HBe	–	–
10	55F[d]	Survived	+	Anti-HBe	–	–
11	70F	Died	+	HBeAg	–	3,040
12	66M[d]	Died	+	Anti-HBe	–	1,797
13	46M[d]	Died	+	Anti-HBe	–	811

[a]In sera diluted 1000-fold.
[b]Tested at the onset of coma.
[c]Developed after the withdrawal of steroid.
[d]Developed after the withdrawal of chemotherapy.

by precore mutations in five (38%) patients with chronic infection (Cases 1–5). In two patients (Cases 6 and 7), core promoter mutations were detected in all clones, of which only about one half possessed the precore mutation for a stop codon.

Patterns of Core Promoter Mutations

Mutations in the core promoter observed in the six patients with acute HBV infection and 12 patients with chronic infection are shown in Figure 2. The double mutation, from A to T at nt 1762 and from G to A at nt 1764, was detected in all clones from the 18 patients. There are three AT-rich regions in the core promoter [Okamoto et al., 1994; Sato et al., 1995], which have been implicated in binding with liver-enriched factors [Lopez-Cabrera et al., 1990] for initiating the transcription of mRNAs by host RNA polymerase. The A-to-T mutation at nt 1762 involved the last A in the second AT-rich region that has been suggested for the transcription of shorter precore mRNAs [Okamoto et al., 1994; Sato et al., 1995]; they have transcription start sites at 26–28 bp downstream of it (Fig. 1).

DISCUSSION

Symptoms of FHF in the 11 patients with acute HBV infection, who had high IgM anti-HBc titres in serum, were more severe and rapid than those in the 13 patients who developed the disease while they carried HBV. Coma occurred sooner after the recognition of jaundice, and transaminases were higher in the patients with acute than chronic HBV infection. A prominent feature not stressed before was a single ALT peak in the majority of patients with acute infection which was in sharp contrast to multiple bouts of ALT elevations in all patients with chronic infection. A single episode of liver damage in the patients with acute HBV infection, represented by only one ALT peak, would be relevant to more severe symptoms of hepatitis. Once the episode was resolved, however, the patients with acute HBV infection appeared to have a better chance of recovery than those with chronic infection.

The patients with chronic infection seemed not to be able to eliminate HBV after the episode of FHF; hepatitis continued to smoulder, which was reflected in subsequent multiple bouts of elevated ALT. Thus, the artificial liver support system involving plasma exchange and haemodiafiltration with high performance membranes [Yoshiba et al., 1993] would have been particularly beneficial for the patients with FHF occurring after acute HBV infection, to overcome a single episode of massive liver damage. This view would be supported by a survival rate somewhat higher in the patients with acute than chronic HBV infection (73% vs. 39%).

O'Grady et al. [1993] proposed to classify FHF by the

TABLE III. Mutations in the Core Promoter and Precore Region of HBV DNA Clones from Patients with Fulminant Hepatic Failure

Patient number	Age and sex	HBeAg or anti-HBe	Clones tested *n*	Mutations in Core promoter[a] *n* (%)	Mutations in Precore region[b] *n* (%)
After Acute HBV Infection					
1	21F	Anti-HBe	24	24 (100%)	24 (100%)
2	38F	Anti-HBe	3	3 (100%)	3 (100%)
3	43M	Anti-HBe	16	16 (100%)	16 (100%)
4	45F	Anti-HBe	5	5 (100%)	5 (100%)
5	47M	Anti-HBe	19	19 (100%)	19 (100%)
6	51M	Anti-HBe	5	5 (100%)	5 (100%)
7	54M	Anti-HBe	5	0	5 (100%)
8	53M	Anti-HBe	5	0	5 (100%)
9	67M	Anti-HBe	16	0	16 (100%)
10	82M	Anti-HBe	10	0	10 (100%)
11	70M	HBeAg	8[c]	0	0
During Chronic HBV Infection					
1	31M	HBeAg	10	10 (100%)	0
2	35M	HBeAg	3	3 (100%)	0
3	40M	Anti-HBe	5	5 (100%)	0
4	52F	HBeAg	6	6 (100%)	0
5	54F	HBeAg	10	10 (100%)	0
6	39M	Anti-HBe	8	8 (100%)	4 (50%)
7	31M	HBeAg	5	5 (100%)	3 (60%)
8	41M	Anti-HBe	3	3 (100%)	3 (100%)
9	46M	Anti-HBe	17	17 (100%)	17 (100%)
10	55F	Anti-HBe	18	18 (100%)	18 (100%)
11	70F	HBeAg	3	3 (100%)	3 (100%)
12	66M	Anti-HBe	3	3 (100%)	3 (100%)
13	46M	Anti-HBe	8	0	8 (100%)

[a]Patterns are shown in Fig. 2.
[b]A point mutation from G to A at nucleotide 1896 converting codon 28 for tryptophan (TGG) to a stop codon (TAG).
[c]One clone had a 7-bp insertion of CATTTTT after nucleotide 1825 to induce a frame shift in the precore region.

duration from jaundice until encephalopathy develops. They define a period of 7 days or less as hyperacute and that of longer than 4 weeks as subacute; and that in between is classified as acute. The survival rate was best for the patients with hyperacute FHF at 36% compared with 7% and 14%, respectively, for those with acute and subacute FHF. Takahashi and Shimizu [1991] reported 29.6% of the patients with FHF with symptoms less than 11 days before encephalopathy survived, in contrast to 14.8% of those with a longer duration. Our results corroborate the concept proposed in these earlier reports.

HBsAg was detected in only six of 11 (55%) patients with acute HBV infection, and IgM anti-HBc in high titres was the only means of diagnosing FHF in the remaining five [Shimizu et al., 1983], contrasting with the detection of HBsAg in all patients with chronic HBV infection. Taken along with a higher survival rate of the patients with acute than chronic HBV infection, these observations would be in agreement with a better survival rate of the patients without detectable HBsAg than those with it (47% vs. 17%) reported by Bernuau et al. [1986]. The replicative activity of HBV, however, was low in patients with either acute or chronic infection. Although HBV DNA was detectable by PCR in all patients with FHF, HBsAg-associated DNA polymerase was not detected in any patient with acute infection, and in only six (46%) patients with chronic infection; the patients with chronic HBV infection had possessed much higher activity for the DNA polymerase before they developed FHF. A low activity of HBV replication has been reported in the patients with FHF who are presumed to have acute HBV infection [Brechot et al., 1984; De Cock et al., 1986].

HBsAg was lost in five of the six patients with acute infection who had possessed it at the onset of FHF. The single patient with HBsAg throughout the course had been on maintenance haemodialysis. Since heightened immune responses are implicated in the pathogenesis of FHF [Trepo et al., 1976; Woolf et al., 1976], compromised immune responses inherent in haemodialysis patients [Goldblum and Reed, 1980] would have been responsible for the persistence of HBsAg in this patient. Six patients with chronic infection developed FHF after the withdrawal of steroid or chemotherapy for the treatment of non-Hogdkin lymphoma. The development of FHF in this situation has been documented [Tassopoulos et al., 1986], and would be attributed to rebounding immune responses to HBV antigens that are suppressed during treatment.

HBV variants with mutations in the precore region to abort the synthesis and secretion of HBeAg, typically the G-to-A point mutation at nt 1896 converting the codon 28 in the precore region to a stop codon [Carman et al., 1989; Okamoto et al., 1990], have been reported in many patients with FHF [Kosaka et al., 1991; Liang et al., 1991]. Of the 11 patients who developed FHF after acute HBV infection, ten (91%) were infected with the precore mutant with the stop codon 28. It was detected in only eight of the 13 (62%) patients who developed FHF while they carried HBV. All HBV DNA clones from the remaining five patients with chronic infection, however, had mutations in the core promoter.

The precore region and the C gene are transcribed into precore mRNAs for the translation of the HBeAg precursor [Bruss and Gerlich, 1988; Garcia et al., 1988]. Hence, mutations interfering with the transcription of precore mRNAs would suppress the synthesis of HBeAg at the transcription level. In actuality, mutations in the second AT-rich region in the core promoter, which would bind with liver-enriched factors [Lopez-Cabrera et al., 1990] for initiating the transcription of precore mRNAs, occur frequently in HBV carriers who seroconvert from HBeAg to anti-HBe as well as in the patients with FHF who are not infected with precore mutants [Okamoto et al., 1994; Sato et al., 1995].

Buckwold et al. [1996] have demonstrated the effect of core promoter mutations on the production of HBeAg in an expression system with cultured cells. They found that the double mutation in the second AT-rich region of the core promoter (T1762 and A1764), reported in the patients with FHF [Sato et al., 1995] and observed also in the patients in this study, prevents the binding of HBV DNA with liver-enriched transcription factors. As a result, the double mutation decreases the tran-

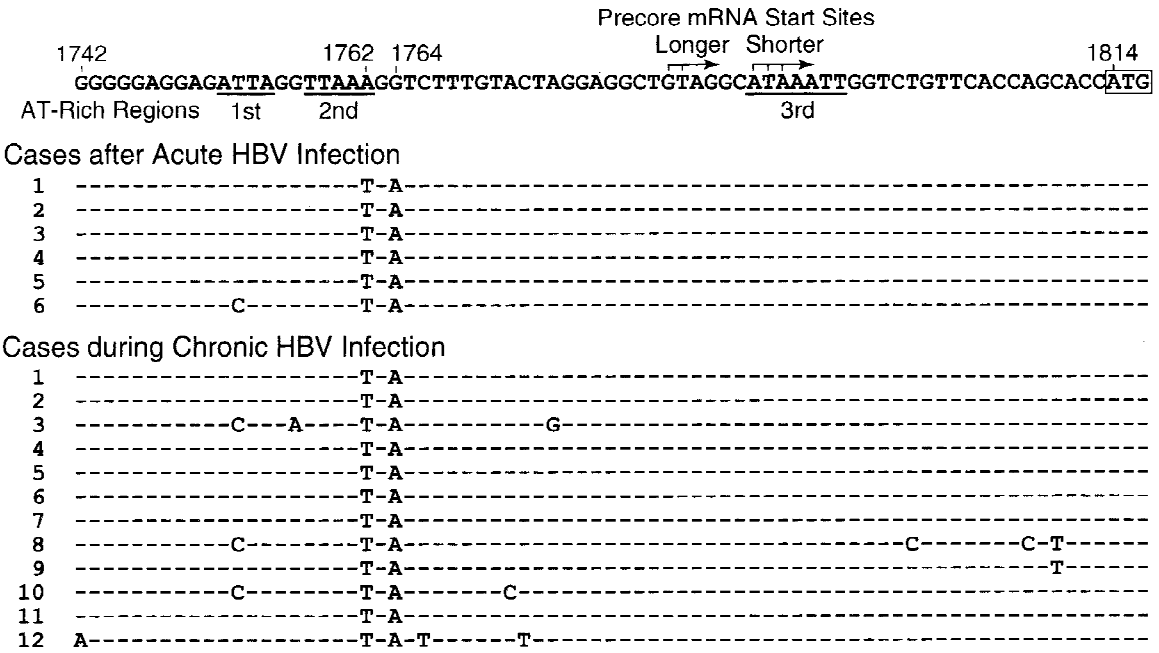

Fig. 2. Point mutations in the core promoter of HBV DNA clones from patients with fulminant hepatic failure. Core promoter mutations were observed in clones from six cases of fulminant hepatic failure occurring after acute HBV infection and in those from 12 of the 13 cases developing during chronic HBV infection. The sequence of a wild-type HBV is indicated above. Three AT-rich regions are underlined by bars and transcription start sites for longer and shorter precore mRNAs by arrows. The initiation codon of the precore region is boxed.

scription of precore mRNAs and expression of HBeAg to approximately one third of that in the wild type. The reduction of precore mRNA transcription is accompanied by an increase in progeny virus production.

Those results reinforce the report of Lamberts et al. [1993] who found that a site-directed mutagenesis for A1896 for the stop codon 28 in the precore region enhances strongly the yield of progeny DNA. Precursor of HBeAg makes an hybrid with the core protein and can inhibit the encapsidation of HBV pregenome, which is implicated in an inhibitory activity of HBeAg on the replication of HBV [Scaglioni et al., 1997]. A separate control of precore mRNAs and the viral pregenome by the core promoter has been demonstrated in expression systems [Yu and Mertz, 1996], which provides a theoretical basis for high replicative activity of HBV variants with mutations in core promoter and incapable of directing a proper synthesis of HBeAg.

Taken together, the lack or decreased production of HBeAg would enhance the replication of HBV, which might account for the induction of FHF by HBV variants with mutations in the precore region, core promoter or both. Since HBeAg can attract host immune responses directed to HBeAg determinant on hepatocytes [Brunetto et al., 1991], its absence would be another factor for a severe disease in the patients infected with HBV variants of an HBeAg-minus phenotype.

The majority of patients with FHF in Europe and the United States are not infected with precore mutants [Feray et al., 1993; Laskus et al., 1993] which would be attributable to HBV of genotype A which is prevalent there and prohibiting the mutation for A1896 [Li et al., 1993]. It would be worthwhile to sequence the core promoter of HBV DNA from these patients to search for any mutations involving the second AT-rich region of the core promoter, although a recent report failed to detect such mutations in HBV DNA clones from patients with FHF in the United States [Laskus et al., 1995].

HBV DNA clones from all the 13 patients with chronic infection were found to have mutations either in the precore region, core promoter, or both. Such mutations would have promoted seroconversion to anti-HBe in them. FHF in these patients would have been an exaggerated manifestation of hepatic damage induced by humoral and cellular immune responses against hepatocytes harboring HBeAg. The validity of such an assumption would need to be evaluated by following for HBV mutants with mutations in the precore region and/or core promoter since before they develop FHF.

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